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APR 19 2002

TECH CENTER 1600/2900

COMMONWEALTH OF AUSTRALIA



IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

-and-

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AUG 27 2003

IN THE MATTER OF: Opposition ~~Opposition by~~ ^{TECH CENTER 1600/2900} Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

STATUTORY DECLARATION

I, Susan Power of Cell & Molecular Technologies, Inc., Phillipsburg, New Jersey, United States of America, declare as follows:

1. At the request of the Patent Attorneys representing Human Genome Sciences ("HGS") in connection with the Ludwig Institute for Cancer Research Opposition to the issuance of HGS Australian Patent Application 696764, in the name of HGS, entitled: "Vascular Endothelial Growth Factor-2" ("the HGS patent specification"), I performed certain experiments as described in a Statutory Declaration executed December 13, 2000 ("Power Declaration I"). The Patent Attorneys representing HGS have now requested that I provide additional information regarding those experiments and carry out additional experiments.
2. In particular, I have been asked to clarify the construction of the expression vectors described in Power Declaration I used to determine whether the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence would result in the expression and secretion of the protein from eukaryotic cells.
3. The Patent Attorneys representing HGS have requested that I perform additional experiments to determine whether the VEGF-2 coding sequence contained in ATCC Deposit No. 75698 fused in frame with a heterologous signal sequence would result in the expression and secretion of the protein

from eukaryotic cells. Further, the Patent Attorneys representing HGS have requested that I construct an expression vector containing the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence using only the VEGF-2 coding sequence contained in the ATCC Deposit No. 75698 and the nucleotide sequence of Figure 1 of the HGS application which contains a nucleotide sequence encoding the 350 amino acid form of VEGF-2, and methods and materials known as of March, 1994. I have done this and the experiments I have conducted are described herein.

The Design and Construction of the Expression Vectors Used in the Experiments Described in Power Declaration I

4. The Patent Attorneys representing HGS had previously asked that I perform experiments in order to determine whether the 350 amino acid form of VEGF-2 would be secreted from cells when attached to a heterologous signal sequence. To achieve this aim, I transfected eukaryotic cells with expression vectors encoding either (1) the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence, or (2) the 419 amino acid form of VEGF-2. The transfected cells were grown and allowed to express the gene products encoded by the vectors. At various time points, both the cell lysates and culture medium were assayed for the presence of VEGF-2 protein. The presence of VEGF-2 protein in either the cell lysates or culture medium was determined by Western Blot analysis of the samples. I have reviewed my notebooks documenting the experiments I performed to achieve the aims of the experiments described in Power Declaration I and provide the following details:
5. For the experiments in Power Declaration I, I was asked to obtain the VEGF-2 DNA directly from the American Tissue Culture Collection (ATCC). I did not obtain any constructs from HGS. The only VEGF-2 clones I obtained were ATCC Deposit No. 97149 and ATCC Deposit No. 75698. The Patent

Attorneys representing HGS provided to me Figure 1 of the HGS patent specification which contains a nucleotide sequence of the 350 amino acid form of VEGF-2. The HGS Patent Attorneys also provided to me the nucleotide sequence of the 419 amino acid form of VEGF-2. It was my understanding that a nucleotide sequence encoding the 350 amino acid form of VEGF-2 was contained in ATCC Deposit No. 75698 and the nucleotide sequence encoding the 419 amino acid form of VEGF-2 was contained in ATCC Deposit No. 97149. It was also my understanding that the amino acid sequence of the 350 amino acid form of VEGF-2 corresponds to residues 70 to 419 of the 419 amino acid form of VEGF-2.

6. As I was under significant time constraints to complete the experiments, I elected to generate the DNA for the expression constructs using only the clone contained in ATCC Deposit No. 97149. Because I was using ATCC Deposit No. 97149 to generate the DNA, I also consulted the nucleotide sequence information relating to the 419 amino acid form of VEGF-2. I considered this to be a reasonable approach since the coding sequences for both the 419 and 350 amino acid forms of VEGF-2 are contained in ATCC Deposit No. 97149. Thus, I isolated the nucleotide sequences encoding the 419 amino acid form of VEGF-2 as well as the 350 amino acid form of VEGF-2 using ATCC Deposit No. 97149 as the sole source of VEGF-2 coding sequences.
7. My understanding of the goals of the experiments described in Power Declaration I was to demonstrate that the 350 amino acid form of VEGF-2 could be successfully expressed and secreted when expressed as taught by the HGS patent specification, *i.e.*, using a heterologous signal sequence. I did not inform the patent attorneys representing HGS at the time of carrying out these experiments nor at the time of signing Power Declaration I that I had isolated the 350 amino acid form of VEGF-2 from the ATCC Deposit No. 97149 clone. It was only when they asked on or about September 24, 2001 for

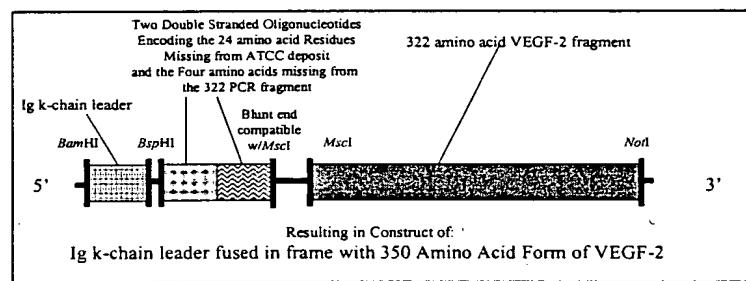
further clarification of the experiments that I conducted that I informed them of these details.

8. I have now been asked to redesign my experimental protocol to specifically use the VEGF-2 coding sequence contained in ATCC Deposit No. 75698 to generate the expression construct containing the 350 amino acid form of VEGF-2. I have been asked that I perform the experiments using the VEGF-2 coding sequence contained in ATCC Deposit No. 75698 to determine whether the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence would result in the expression and secretion of the protein from eukaryotic cells.
9. I have provided the Patent Attorneys for HGS with the details of a sequence analysis of the VEGF-2 coding sequence contained in ATCC Deposit No. 75698. The VEGF-2 clone contained in the ATCC Deposit No. 75698 lacks 24 amino acids at the N-terminal end of the 350 amino acid form of VEGF-2, and corresponds to residues 94 to 419 of the 419 amino acid form of VEGF-2, *i.e.*, a 326 amino acid form of VEGF-2. I have also been asked to perform experiments to determine if the 326 amino acid form of VEGF-2 as encoded by a nucleotide sequence contained in ATCC Deposit No. 75698 fused to a heterologous signal sequence would result in the expression and secretion of the protein from eukaryotic cells.
10. Even though ATCC Deposit No. 75698 lacks the complete coding sequence for the 350 amino acid form of VEGF-2, a molecular biologist as of March 1994 would be able to recreate the 350 amino acid form of VEGF-2 given the description of the complete sequence in the HGS patent specification (as described below) and that is the course I could have taken at that time and I would have expected other molecular biologists to have been able to do the same. I generated an expression vector containing the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence, using only the

ATCC Deposit No. 75698 and the sequence of Figure 1 in the HGS patent specification, and techniques and materials routinely known and used in the art as of March 1994.

The Expression Vector Containing the 350 Amino Acid Form of VEGF-2 Is Generated Using Only ATCC Deposit No. 75698 and Figure 1 of the HGS Patent Specification

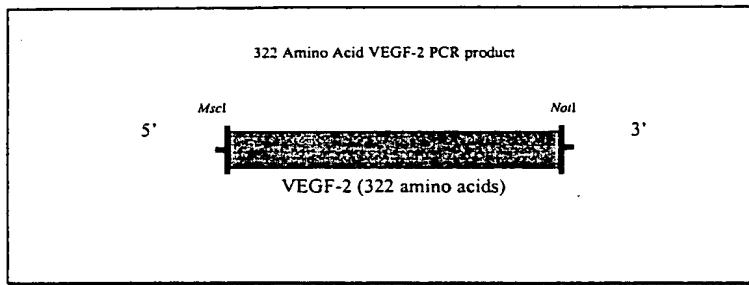
11. The general design of the expression vector containing the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence is as follows:



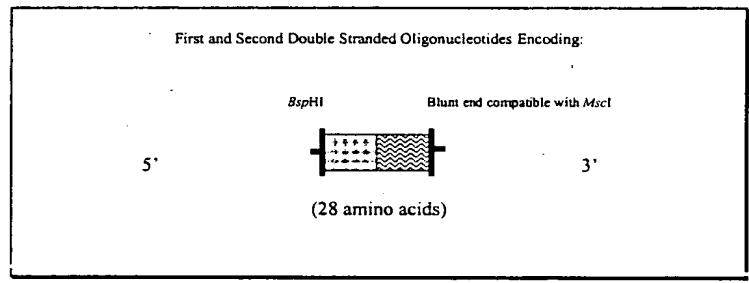
12. Since all that I had at my disposal were ATCC Deposit No. 75698 and Figure 1 of the HGS patent specification, I did the following:

- 12.1 First, I chose to directly isolate a nucleotide sequence encoding the C-terminal 322 residues of the 326 amino acid form of VEGF-2. The 322 residues corresponding to residues 98 to 419 of the 419 amino acid form of VEGF-2 were amplified by PCR from ATCC Deposit No. 75698. I chose to isolate a VEGF-2 fragment of 322 amino acids to facilitate the cloning of the VEGF-2 coding sequence in frame into the expression constructs. To do so, I designed primers based on the sequence provided in Figure 1 of the HGS patent specification, the sequence of ATCC Deposit No. 75698, and the sequence of restriction enzyme recognition sites, e.g., *MscI* and *NotI*. The resulting 322 amino

acid fragment of VEGF-2 amplified from ATCC Deposit No. 75698 was digested with *Msc* I and *Not* I.

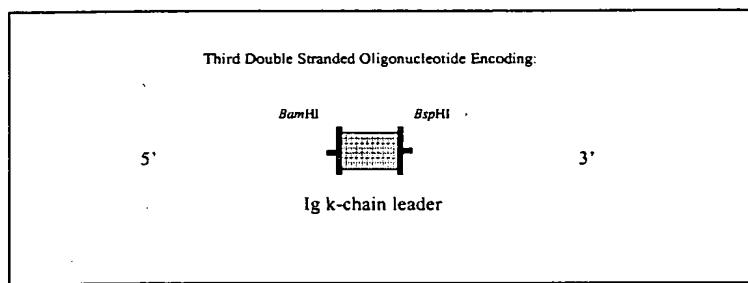


12.2 Using a nucleotide sequence encoding the 350 amino acid form of VEGF-2 contained in Figure 1 of the HGS specification, I designed two double stranded oligonucleotides to encode (once ligated together) a 28 amino acid VEGF-2 fragment. This fragment encompasses the 24 amino acids missing from ATCC Deposit No. 75698 and the additional 4 amino acids missing from the 322 amino acid fragment of the 326 form of VEGF-2. Specifically, once ligated together, the oligonucleotides were designed to result in the generation of a 28 amino acid fragment engineered to have a 5' end with a *Bsp*H I restriction site overhang and a 3' blunt end compatible with a *Msc* I restriction site as shown below. Methods and materials for generating such double stranded oligonucleotides were routine and known by March, 1994.



12.3 A third double stranded oligonucleotide encoding the secretion signal sequence of the Ig k-chain leader signal sequence that was also used in

the experiments described in Power Declaration I was engineered to contain a *Bam* HI restriction enzyme overhang at the 5' end and a *Bsp* HI restriction enzyme overhang at the 3' end as shown below. Ig k-chain leader signal sequence was a recognized signal sequence available as of March, 1994.



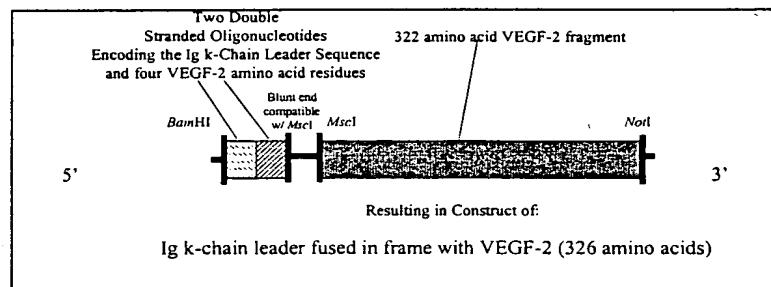
12.4 The 322 amino acid VEGF-2 fragment and the three double stranded oligonucleotides described above were ligated and subcloned at once into the *Not I/Bam HI* sites of the expression vector pCMV-I which was described in Power Declaration I. The resulting expression vector contains the construct as described in ¶ 11 above. The VEGF-2 sequence is under the control of a CMV-I promoter, a promoter routinely used as of March, 1994.

12.5 The resulting 350 amino acid form of VEGF-2 construct was sequenced and confirmed to be correct and is detailed in Appendix I.

13. The design of the expression vector containing the VEGF-2 coding sequence found in ATCC Deposit No. 75698 used in the study results in a construct with the 326 amino acid form of VEGF-2 linked to a heterologous sequence and is as follows:

14. To generate the construct, the 322 amino acid VEGF-2 fragment flanked with a *Msc I* site at the 5' end and the *Not I* site at the 3' end was generated as

described above (see ¶12.1). I designed two double stranded oligonucleotides that once ligated together encoded the Ig k-chain leader signal sequence and the four amino acid residues corresponding to residues 94 to 97 of the 419 amino acid form of VEGF-2, *i.e.*, the first four residues of the 326 amino acid form of VEGF-2 of ATCC Deposit No. 75698 engineered to contain a 3' blunt end compatible with a *MscI* restriction site and a 5' *Bam* HI site. The 322 amino acid VEGF-2 fragment was simultaneously fused in frame with the two double stranded oligonucleotides, as shown below, and subcloned into the expression vector pCMV-I *Bam* HI/ *Not* I sites. Again, the VEGF-2 sequence is under the control of a CMV-I promoter, a promoter routinely used as of March 1994.



15. The sequence of the resulting 326 amino acid form of VEGF-2 construct was confirmed to be correct and is detailed in Appendix II, attached hereto.
16. For purposes of the following experiments, I used the expression vector encoding the 419 amino acid form of VEGF-2 described in Power Declaration I (*see* Power Declaration I ¶¶ 3 to 6).
17. As set out in Power Declaration I, the sequence of the construct was confirmed to be correct and is detailed in Appendix III, attached hereto.

Using Only the VEGF-2 Clone Contained in ATCC Deposit No. 75698 Fused in Frame with a Heterologous Signal Sequence, Expression and Secretion of VEGF-2 Is Achieved

18. The Patent Attorneys for HGS requested that I perform the following experiments in order to determine whether using only the 350 amino acid form or the 326 amino acid form of VEGF-2 contained in ATCC Deposit No. 75698 fused in frame with a heterologous signal sequence would result in the expression and secretion of VEGF-2 from eukaryotic cells.
19. The overall experimental design is as follows: eukaryotic cells were transfected with expression vectors encoding the 419 amino acid form of VEGF-2, the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence, or the 326 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence. The transfected cells were cultured for 24 or 48 hours to allow for expression of the gene products encoded by each vector. In order to determine whether the VEGF-2 gene product was being expressed and secreted, the cell lysates and culture medium were collected to assay for the presence of VEGF-2. The presence of VEGF-2 in either the cell lysates or culture medium was determined by Western Blot analysis of the samples using a rabbit polyclonal antibody to VEGF-2 that recognizes all forms and fragments of VEGF-2. The same antibody was used in Power Declaration I to assay the presence of VEGF-2 proteins.
20. The three VEGF-2 constructs encoding the 419, 350 and 326 amino acid forms of VEGF-2 each were transiently transfected in duplicate, using the lipofectin method into the Human Embryonic Kidney cell line, HEK-293 tsA-0. The method of transfection and the cell line were both routinely used as of March, 1994. As a control for transfection efficiency, each construct was co-transfected with the vector pCMV- β -gal. The efficiency of transfection was determined by β -gal staining 48 hours after transfection. As a negative control,

the vector pCMV-I without the addition of any VEGF-2 coding sequences was transfected in parallel.

21. The transfection design is as follows:

6 dishes transfected with: pCMV-I-VEGF-419;

6 dishes transfected with: pCMV-I-signal sequence-VEGF-350;

6 dishes transfected with: pCMV-I-signal sequence-VEGF-326;

6 dishes transfected: pCMV-I;

1 dish transfected with: pCMV-I-VEGF-419 + pCMV- β -gal;

1 dish transfected with: pCMV-I-signal sequence-VEGF-350 + pCMV- β -gal; and

1 dish transfected with: pCMV-I-signal sequence-VEGF-326 + pCMV- β -gal.

22. After transfection, DMEM medium containing 3% serum was added to the cells. Aliquots of cell extracts and conditioned medium were prepared from each transfection at: T_0 hours, T_{24} hours and T_{48} hours, in duplicate.

23. At the time of harvesting the cells and medium were treated as follows:

Medium: Harvested medium was concentrated 3 fold using Centricon 10 concentrator devices. One volume of 2 x PAGE loading dye was added to each sample.

Cell Extracts: The cells were harvested by trypsinization and collected by centrifugation. The cell pellet was resuspended and lysed, and one volume of 2 x PAGE loading dye was added to each sample.

24. To determine the transfection efficiency, dishes transfected with the pCMV- β -gal construct were fixed and stained for β -gal activity 48 hours after transfection. All dishes were found to have the same percentage of transfected cells (70%).

25. Each protein sample was subjected to Western analysis as outlined below. Prior to loading on to a 12% (w/v) Tris-Glycine SDS-Polyacrylamide gel, the samples were boiled for 5 minutes and cooled on ice. The two end lanes of each gel contained the appropriately sized molecular weight markers to estimate the migration rate of proteins predicted to run in the 16 to 85 kDa size range. The samples were electrophoresed according to standard conditions.

26. Following electrophoresis, the samples were transferred to a PVDF membrane. Each membrane was blocked by a one hour incubation in phosphate buffered saline (PBS) containing 3% Bovine Serum Albumin (BSA). The blot was then incubated at 4°C in PBS containing 0.1% BSA and 500ng/ml of purified rabbit anti-VEGF-2 antibody, a polyclonal antibody which recognizes all immunogenic fragments of VEGF-2. After three 5 minute washes in PBS containing 0.1% Tween, the blot was incubated for 1 hour in PBS containing 0.1% BSA and a 1:3000 dilution of Goat Anti-Rabbit IgG Horse Radish Peroxidase (HRP) conjugated antibody. The blot was washed six times for 5 minutes in PBS containing 0.1% BSA. The blot was developed with 2ml/blot of ECL detection reagent (obtained from Amersham) for one minute and then exposed directly to Polaroid films for approximately 2-3 seconds.

27. The result of the experiment is shown in Figure 1, attached hereto. The samples included in the figure are as follows:

Immunoblot analysis of VEGF constructs transiently expressed in HEK293T cells

Lane	Pellet/ Supernatant	Construct (419, 350, 326, or neg. control)	T (h) post-transfection
Gel 1			
1	P	Negative control	24
2	S	Negative control	24
3	P	350-signal	24
4	S	350-signal	24
5	P	326-signal	24

6	S	326-signal	24
7	P	419	48
8	S	419	48

Gel 2

1	P	Negative control	24
2	S	Negative control	24
3	P	350-signal	24
4	S	350-signal	24
5	P	326-signal	24
6	S	326-signal	24
7	P	419	48
8	S	419	48

Gel 3

1	P	Negative control	24
2	S	Negative control	24
3	P	419	24
4	S	419	24
5	P	419	24
6	S	419	24
7	P	350-signal	48
8	S	350-signal	48
9	P	419	48
10	S	419	48

Gel 4

1	P	Negative control	48
2	S	Negative control	48
3	P	350-signal	48
4	S	350-signal	48
5	P	326-signal	48
6	S	326-signal	48
7	P	326-signal	48
8	S	326-signal	48

9	P	419	48
10	S	419	48

31. The Western Blot analysis indicates a broad band resolving at approximately 30kDa was present in the medium collected from the transfection of the 419 amino acid VEGF-2 construct, the 350 amino acid VEGF-2 signal sequence construct, and the 326 amino acid VEGF-2 signal sequence construct (see Figure 1, attached hereto as Appendix IV). The secreted protein was visible at 24 hours and 48 hours after transfection. The secreted product from cells containing the 419 amino acid VEGF-2 construct, the 350 amino acid VEGF2 signal sequence construct, and the 326 amino acid VEGF-2 signal sequence construct are all the same approximate size.

AND I declare that all the statements made in this Declaration are of my own are true in every particular, and that all statements made on information and belief are believed to be true.

Sworn by the said Susan Power, Susan Power at
 Phillipsburg, New Jersey, on this 22 day of March 2002;
 before me Gean Rotmistrenko
 Notary Public

GEAN ROTMISTRENKO
 Notary Public, State of New York
 No. 41-4778718
 Qualified in Queens County
 Certificate Filed in New York County
 Commission Expires October 31, 2025

VEGF-350+Signal

	BamHI	Ncol																	
1	Met	Glu	Thr	Asp	Thr	Leu	Leu	Leu	Trp	Val	Leu	Leu	Leu						
1	GGATCCGCCA	CCATGGAGAC	AGACACACTC	CTGCTATGGG	TACTGCTGCT														
	CCTAGGCGGT	GGTACCTCTG	TCTGTGTGAG	GACGATACCC	ATGACGACGA														
+1	Leu	Trp	Val	Pro	Gly	Ser	Thr	Gly	Asp	Met	Thr	Val	Leu	Tyr	Pro	Glu	Tyr	Trp	
51	CTGGGTTCCA	GGTTCCACTG	GTGACATGAC	TGTACTCTAC	CCAGAATATT														
	GACCCAAGGT	CCAAGGTGAC	CACTGTACTG	ACATGAGATG	GGTCTTATAA														
+1	Trp	Lys	Met	Tyr	Lys	Cys	Gln	Leu	Arg	Lys	Gly	Gly	Trp	Gln	His	Asn	Arg		
101	GGAAAATGTA	CAAGTGTAG	CTAAGGAAAG	GAGGCTGGCA	ACATAACAGA														
	CCTTTACAT	GTTCACAGTC	GATTCCCTTC	CTCCGACCGT	TGTATTGTCT														
+1	Glu	Gln	Ala	Asn	Leu	Asn	Ser	Arg	Thr	Glu	Glu	Thr	Ile	Lys	Phe	Ala	Ala		
151	GAACAGGCCA	ACCTCAACTC	AAGGACAGAA	GAGACTATAA	AATTGCTGCT														
	CTTGTCCGGT	TGGAGTTGAG	TTCCGTCTT	CTCTGATATT	TTAACGACG														
	BglII																		
+1	Ala	Ala	His	Tyr	Asn	Thr	Glu	Ile	Leu	Lys	Ser	Ile	Asp	Asn	Glu	Trp	Arg	Lys	
201	AGCACATTAT	AATACAGAGA	TCTTGAAGAAG	TATTGATAAT	GAGTGGAGAA														
	TCGTGTAATA	TTATGTCTCT	AGAACTTTTC	ATAACTATTA	CTCACCTCTT														
	SphI																		
+1	Lys	Thr	Gln	Cys	Met	Pro	Arg	Glu	Val	Cys	Ile	Asp	Val	Gly	Lys	Glu	Phe		
251	AGACTCAATG	CATGCCACGG	GAGGTGTGTA	TAGATGTGGG	GAAGGGAGTT														
	TCTGAGTTAC	GTACGGTGCC	CTCCACACAT	ATCTACACCC	CTTCCCTCAAA														
	DraI																		
+1	Gly	Val	Ala	Thr	Asn	Thr	Phe	Phe	Lys	Pro	Pro	Cys	Val	Ser	Val	Tyr	Arg		
301	GGAGTCGCGA	CAAACACCTT	CTTAAACCT	CCATGTGTGT	CCGTCTACAG														
	CCTCAGCGCT	TTTGTTGAA	GAAATTGGA	GGTACACACA	GGCAGATGTC														
+1	Arg	Cys	Gly	Gly	Cys	Cys	Cys	Asn	Ser	Glu	Gly	Leu	Gln	Cys	Met	Asn	Thr	Ser	Thr
351	ATGTGGGGGT	TGCTGCAATA	GTGAGGGGCT	GCAGTGCATG	AACACCAGCA														
	TACACCCCCA	ACGACGTTAT	CACTCCCCGA	CGTCACGTAC	TTGGGGTCGT														
+1	Thr	Ser	Tyr	Leu	Ser	Lys	Thr	Leu	Phe	Glu	Ile	Thr	Val	Pro	Leu	Ser	Gln		
401	CGAGCTACCT	CAGCAAGACG	TTATTGAAA	TTACAGTGCC	TCTCTCTCAA														
	GCTCGATGGA	GTCGTTCTGC	AATAAACTTT	AATGTCACCGG	AGAGAGAGTT														
+1	Gly	Pro	Lys	Pro	Val	Thr	Ile	Ser	Phe	Ala	Asn	His	Thr	Ser	Cys	Arg	Cys		
451	GGCCCCAAAC	CAGTAACAAAT	CAGTTTGCC	AATCACACTT	CCTGCCGATG														
	CCGGGGTTTG	GTCATTGTTA	GTCAAAACGG	TTAGTGTGAA	GGACGGCTAC														
+1	Cys	Met	Ser	Lys	Leu	Asp	Val	Tyr	Arg	Gln	Val	His	Ser	Ile	Ile	Arg	Arg	Ser	
501	CATGTCTAAA	CTGGATGTTT	ACAGACAACT	TCATTCCATT	ATTAGACGTT														
	GTACAGATT	GACCTACAAA	TGTCTGTTCA	AGTAAGGTAA	TAATCTGCAA														
+1	Ser	Leu	Pro	Ala	Thr	Leu	Pro	Gln	Cys	Gln	Ala	Ala	Asn	Lys	Thr	Cys	Pro		
551	CCCTGCCAGC	AACACTACCA	CAGTGTCAAG	CAGCGAACAA	GACCTGCC														
	GGGACGGTCG	TTGTGATGGT	GTCAAGTCC	GTGGCTTGT	CTGGACGGGG														
+1	Thr	Asn	Tyr	Met	Trp	Asn	Asn	His	Ile	Cys	Arg	Cys	Leu	Ala	Gln	Glu	Asp		
601	ACCAATTACA	TGTGGAATAA	TCACATCTGC	AGATGCCCTGG	CTCAGGAAGA														
	TGGTTAATGT	ACACCTTATT	AGTGTAGACG	TCTACGGACC	GAGTCCTTCT														
+1	Asp	Phe	Met	Phe	Ser	Ser	Asp	Ala	Gly	Asp	Asp	Ser	Thr	Asp	Gly	Phe	His	Asp	
651	TTTTATGTTT	TCCTCGGATG	CTGGAGATGA	CTCAACAGAT	GGATTCCATG														
	AAAATACAAA	AGGAGCCTAC	GACCTCTACT	GAGTTGTCTA	CCTAAGGTAC														

POWER DECLARATION II

APPENDIX I

VEGF-350+Signal

1 Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val
 701 ACATCTGTGG ACCAAACAAG GAGCTGGATG AAGAGACCTG TCAGTGTGTC
 TGTAGACACC TGGTTTGTTC CTCGACCTAC TTCTCTGGAC AGTCACACAG
 BsrBI
 1 Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu Asp
 751 TGCAGAGCGG GGCTTCGCC TGCCAGCTGT GGACCCCCACA AAGAACTAGA
 ACGTCTGCC CCGAAGCCGG ACGGTCGACA CCTGGGGTGT TTCTTGATCT
 1 Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Ser Gln Cys
 801 CAGAAACTCA TGCCAGTGTG TCTGTAAAAA CAAACTCTTC CCCAGCCAAT
 GTCTTGAGT ACGGTACACAG ACACATTTT GTTGAGAAG GGGTCGGTTA
 1 Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys Lys
 851 GTGGGGCCAA CCGAGAATTG GATGAAAACA CATGCCAGTG TGATGTAAA
 CACCCCGGTT GGCTCTAAA CTACTTTGT GTACGGTCAC ACATACATT
 1 Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala Cys Glu
 901 AGAACCTGCC CCAGAAATCA ACCCCTAAAT CCTGGAAAAT GTGCCGTG
 TCTGGACGG GGTCTTAGT TGGGGATTAA GGACCTTTA CACGGACACT
 1 Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly Lys Lys Phe His His
 951 ATGTACAGAA AGTCCACAGA AATGCTTGT AAAAGGAAAG AAGTTCCACC
 TACATGTCCT TCAGGTGTCT TTACGAACAA TTTCTCTTC TTCAAGGTGG
 1 His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Ds Thr Asn Arg Gln Lys Ala
 1001 ACCAAACATG CAGCTGTTAC AGACGGCCAT GTACGAACCG CCAGAAGGCT
 TGGTTTGTAC GTCGACAATG TCTGCCGGTA CATGCTTGGC GGTCTTCCGA
 1 Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser
 1051 TGTGAGCCAG GATTTCTATA TAGTGAAGAA GTGTGTCGTT GCGTCCCTTC
 ACACCTGGTC CTAAAAGTAT ATCACTTCTT CACACAGCAA CGCAGGGAAAG
 NotI
 EagI
 1 Ser Tyr Trp Lys Arg Pro Gln Met Ser —
 1101 ATATTGGAAA AGACCACAAA TGAGCTAACG GGCGCG
 TATAACCTT TCTGGTGTTC ACTCGATTG CGCGCGC

VEGF 326+Signal

	BamHI	Ncol
1	Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu	
1	GGATCCGCCA CCATGGAGAC AGACACACTC CTGCTATGGG TACTGCTGCT	
	CCTAGGCGGT GGTACCTCTG TCTGTGTGAG GACGATAACCC ATGACGACGA	
+1	Leu Trp Val Pro Gly Ser Thr Gly Asp Arg Glu Gln Ala Asn Leu Asn Ser Arg	
51	CTGGGTTCCA GGTTCCACTG GTGACAGAGA ACAGGCCAAC CTCAACTCAA	
	GACCCAAGGT CCAAGGTGAC CACTGTCTCT TGTCCGGTTG GAGTTGAGTT	
	BglII	
+1	Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile	
101	GGACAGAAGA GACTATAAAA TTTGCTGCAG CACATTATAA TACAGAGATC	
	CCTGTCTTCT CTGATATTTT AAACGACGTC GTGTAATATT ATGTCTCTAG	
	SphI	
+1	Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu	
151	TTGAAAAGTA TTGATAATGTA GTGGAGAAAG ACTCAATGCA TGCCACGGGA	
	AACTTTCAT AACTATTACT CACCTCTTTC TGAGTTACGT ACGGTGCCCT	
	DraI	
+1	Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val Ala Thr Asn Thr Phe Phe	
201	GGTGTGTATA GATGTGGGGA AGGAGTTGG AGTQGCCGACA AACACCTTCT	
	CCACACATAT CTACACCCCT TCCTCAAACC TCAGCGCTGT TTGTGGAAGA	
	DraI	
+1	Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn Ser	
251	TTAAACCTCC ATGTGTGTCC GTCTACAGAT GTGGGGGTTG CTGCAATAGT	
	AATTGGAGG TACACACAGG CAGATGTCTA CACCCCCAAC GACGTTATCA	
+1	Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu Ser Lys Thr Leu	
301	GAGGGGCTGC AGTGCATGAA CACCAAGCAG AGCTACCTCA GCAAGACGTT	
	CTCCCCGACG TCACGTACTT GTGGTCGTGC TCGATGGAGT CGTTCTGCAA	
+1	Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val Thr Ile Ser	
351	ATTTGAAATT ACAGTGCCTC TCTCTCAAGG CCCCCAAACCA GTAACAATCA	
	TAAACTTAA TGTACCGGAG AGAGAGTTCC GGGGTTTGGT CATTGTTAGT	
+1	Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp Val Tyr	
401	GTGTTGCCAA TCACACTTCC TGCCGATGCA TGTCTAAACT GGATGTTTAC	
	CAAACGGTT AGTGTGAAGG ACGGCTACGT ACAGATTGAA CCTACAAATG	
+1	Arg Gin Val His Ser Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gin	
451	AGACAAGTTTC ATTCCATTAT TAGACGTTCC CTGCCAGCAA CACTACCACA	
	TCTGTTCAAG TAAGGTAATA ATCTGCAAGG GACGGTCGTT GTGATGGTGT	
+1	Gly Cys Gin Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His	
501	GTGTCAGGCA GCGAACAAAGA CCTGCCAAC CAATTACATG TGGAAATAATC	
	CACAGTCCGT CGCTTGTCT GGACGGGGTG GTTAATGTAC ACCTTATTAG	
+1	His Ile Cys Arg Cys Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala	
551	ACATCTGCAG ATGCCCTGGCT CAGGAAGATT TTATGTTTTC CTGGGATGCT	
	TGTAGACGTC TACGGACCGA GTCCCTCTAA AATACAAAAG GAGCCTACGA	
+1	Gly Asp Asp Ser Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu	
601	GGAGATGACT CAACAGATGG ATTCCATGAC ATCTGTGGAC CAAACAAGGA	
	CCTCTACTGA GTTGTCTACC TAAGGTACTG TAGACACCTG GTTGTCTCT	
	BsrBI	
+1	Gln Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala	
651	GCTGGATGAA GAGACCTGTC AGTGTGTCTG CAGAGCGGGG CTTCCGGCCTG	
	CGACCTACTT CTCTGGACAG TCACACAGAC GTCTCGCCCC GAAGCCGGAC	

POWER DECLARATION II

APPENDIX II

VEGF 326+Signal

+1 Ala Ser Cys Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gin Cys Val
 701 CCAGCTGTGG ACCCCCACAAA GAACTAGACA GAAACTCATG CCAGCTGTGTC
 GGTGACACCC TGGGGTGTCTT CTTGATCTGT CTTTGAGTAC GGTGACACAG
 +1 Cys Lys Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp
 751 TGTAAAAACA AACTCTTCCC CAGCCAATGT GGGGCCAAC GAGAATTGAG
 ACATTTTGT TTGAGAAGGG GTCGGTTACA CCCCCGGTTGG CTCTTAAACT
 +1 Asp Glu Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro
 801 TGAAAACACA TGCCAGTGTG TATGTAAAAG AACCTGCCCC AGAAATCAAC
 ACTTTGTGT ACGGTACAC ATACATTTC TTGGACGGGG TCTTTAGTTG
 EsrGI
 +1 Pro Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gin Lys
 851 CCCTAAATCC TGGAAAATGT GCCTGTGAAT GTACAGAAAG TCCACAGAAA
 GGGATTTAGG ACCTTTACA CGGACACTTA CATGTCTTTC AGGTGTCTT
 +1 Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg
 901 TGCTGTAA AAGGAAAGAA GTTCCACCAAC CAAACATGCA GCTGTACAG
 ACGAACAAATT TTCCCTTCTT CAAGGTGGTG GTTGTACGT CGACAATGTC
 +1 Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser
 951 ACGGCCATGT ACGAACCGCC AGAAGGCTTG TGAGCCAGGA TTTTCATATA
 TGCCGGTACA TGCTTGGCGG TCTTCCGAAC ACTCGGTCTT AAAAGTATAT
 +1 Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gin Met
 1001 GTGAAGAAGT GTGTCGTTGT GTCCCTTCAT ATTGGAAAAG ACCACAAATG
 CACTTCTTCA CACAGCAACA CAGGGAAGTA TAACCTTTTC TGGTGTTCAC
 NotI
 EagI
 +1 Ser **
 1051 AGCTAAGCGG CCGCG
 TCGATTGCC GGCAC

EcoRI

+1 Met His Leu Leu Gly Phe Phe Ser Val Ala
 1 GAATTCGTGG GTCCTTCCAC CATGCACTTG CTGGGCTTCT TCTCTGTGGC
 CTTAAGCACC CAGGAAGGTG GTACGTGAAC GACCCGAAGA AGAGACACCG
 SmaI
 XmaI
 Aval Nael
 +1 Ala Cys Ser Leu Leu Ala Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala
 51 GTGTTCTCTG CTCGCCGCTG CGCTGCTCCC GGGTCCCTCGC GAGGCGCCCG
 CACAAGAGAC GAGCGGCAC GCGACCGAGGG CCCAGGAGCG CTCCCGCAGGC
 +1 Ala Ala Ala Ala Ala Phe Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro
 101 CCGCCGCCGC CGCCTTCGAG TCCGGACTCG ACCTCTCGGA CGCGGAGGCC
 GGCGCGGGCG GCGGAAGCTC AGGCCTGAGC TGGAGAGCCT GCGCCTCGGG
 +1 Asp Ala Gly Glu Ala Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Glu Leu
 151 GACGCCGGCG AGGCCACGGC TTATGCAAGC AAAGATCTGG AGGAGCAGT
 CTGCGCCCGC TCCGGTGGCG AATACGTTCG TTTCTAGACC TCCTCGTCAA

BspHI

+1 Leu Arg Ser Val Ser Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr
 201 ACGGTCTCTG TCCAGTGTAG ATGAACTCAT GACTGTACTC TACCCAGAAC
 TGCCAGACAC AGGTACACATC TACTTGAGTA CTGACATGAG ATGGGTCTTA
 +1 Tyr Trp Lys Met Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn
 251 ATTGGAAAAT GTACAAGTGT CAGCTAAGGA AAGGAGGCTG GCAACATAAC
 TAACCTTTA CATGTTACA GTCGATTCTT TTCCCTCCGAC CGTTGTATTG
 +1 Arg Glu Gln Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala
 301 AGAGAACAGG CCAACCTCAA CTCAGGACA GAAGAGACTA TAAAATTTGC
 TCTCTGTCC GGTTGGAGTT GAGTTCTGT CTTCTGTAT ATTTAAACG
 +1 Ala Ala Ala His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg
 351 TGCAGCACAT TATAATACAG AGATCTTGAA AAGTATTGAT AATGAGTGGA
 ACGTCGTGTA ATATTATGTC TCTAGAACTT TTCATAACTA TTACTCACCT

SphI

+1 Arg Lys Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu
 401 GAAAGACTCA ATGCATGCCA CGGGAGGTGT GTATAGATGT GGGGAAGGAG
 CTTCTGAGT TACGTACGGT GCCCTCCACA CATATCTACA CCCCTTCCTC
 +1 Phe Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr
 450 TTTGGAGTCG CGACAAACAC CTTCTTTAAA CCTCCATGTG TGTCCGTCTA
 AACACCTCAGC GCTGTTGTG GAAGAAATT GGAGGTACAC ACAGGCAGAT
 AccI
 +1 Tyr Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser
 500 CAGATGTGGG GGTTGCTGCA ATAGTGAGGG GCTGCAGTGC ATGAACACCA
 GTCTACACCC CCAACGACGT TATCACTCCC CGACGTCACG TACTTGTGGT
 +1 Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser
 550 GCACGAGCTA CCTCAGCAAG ACGTTATTG AAATTACAGT GCCCTCTCT
 CGTGCCTCGAT GGAGTCGTTC TGCAATAAAC TTTAATGTCA CGGAGAGAGA
 +1 Glu Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg
 600 CAAGGCCCA AACCAAGTAAC AATCAGTTT GCCAATCACA CTTCCGTGGC
 GTTCCGGGGT TTGGTCATTG TTAGTCAAAA CGGTTAGTGT GAAGGACGGC

+1 Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gin Val His Ser Ile Ile Arg Arg
 651 ATGCATGTCT AAACCTGGATG TTTACAGACA AGTTCAATTCC ATTATTAGAC
 TACGTACAGA TTTGACCTAC AAATGTCTGT TCAAGTAAGG TAATAATCTG
 +1 Arg Ser Leu Pro Ala Thr Leu Pro Gin Cys Gin Ala Ala Asn Lys Thr Cys
 701 GTTCCCTGCC AGCAACACTA CCACAGTGTGTC AGGCAGCGAA CAAGACCTGC
 CAAGGGACGG TCGTTGTGAT GGTGTCACAG TCCGTCGCTT GTTCTGGACG
 +1 Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala Gin Glu
 751 CCCACCAATT ACATGTGGAA TAATCACATC TGCAGATGCC TGGCTCAGGA
 GGGTGGTTAA TGTACACCTT ATTAGTGTAG ACGTCTACGG ACCGAGTCCT
 +1 Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp Gly Phe His
 801 AGATTTTATG TTTTCTCGG ATGCTGGAGA TGACTCAACA GATGGATTC
 TCTAAAATAC AAAAGGAGCC TACGACCTCT ACTGAGTTGT CTACCTAAGG
 +1 His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gin Cys
 851 ATGACATCTG TGGACCAAAC PAGGAGCTGG ATGAAGAGAC CTGTCAGTGT
 TACTGTAGAC ACCTGGTTTG TTCTCGACC TACTTCTCTG GACAGTCACA
 BsrBI
 +1 Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu
 901 GTCTGCAGAG CGGGGCTTCG GCCTGCCAGC TGTGGACCCC ACAAAAGAACT
 CAGACGTCTC GCCCGAAGC CGGACGGTCG ACACCTGGGG TGTTCCTTGA
 +1 Leu Asp Arg Asn Ser Cys Gin Cys Val Cys Lys Asn Lys Leu Phe Pro Ser Gin
 951 AGACAGAAAC TCATGCCAGT GTGCTGTAA AAACAAACTC TTCCCCAGCC
 TCTGTCTTG AGTACGGTCA CACAGACATT TTGTGGAG AAGGGGTCGG
 +1 Glu Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gin Cys Val Cys
 1001 AATGTGGGGC CAACCGAGAA TTGATGAAA ACACATGCCA GTGTGTATGT
 TTACACCCCG GTTGGCTCTT AACTACTTT TGTGTACGGT CACACATACA
 +1 Lys Arg Thr Cys Pro Arg Asn Gin Pro Leu Asn Pro Gly Lys Cys Ala Cys
 1051 AAAAGAACCT GCCCCAGAAA TCAACCCCTA AATCCTGGAA AATGTGCCTG
 TTTCTTGGA CGGGGTCTT AGTTGGGGAT TTAGGACCTT TTACACGGAC
 +1 Cys Glu Cys Thr Glu Ser Pro Gin Lys Cys Leu Leu Lys Gly Lys Lys Phe His
 1101 TGAATGTACA GAAAGTCCAC AGAAATGCTT GTAAAAGGA AAGAAGTTCC
 ACTTACATGT CTTTCAGGGTG TCTTACGAA CAATTTCTT TTCTTCAAGG
 +1 His His Gin Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr Asn Arg Gin Lys
 1151 ACCACCAAAC ATGCAGCTGT TACAGACGGC CATGTACGAA CCGCCAGAAG
 TGGTGGTTTG TACGTGACCA ATGCTCTGCC GTACATGCTT GGCGGTCTTC
 +1 Ala Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro
 1201 GCTTGTGAGC CAGGATTTTC ATATAGTGAAGA GAAGTGTGTC GTTGTGTC
 CGAACACTCG GTCCTAAAAG TATATCACTT CTTCACACAG CAACACAGGG
 NotI
 +1 Pro Ser Tyr Trp Lys Arg Pro Gin Met Ser ***
 1251 TTCAATTGG AAAAGACCAAC AAATGAGCTA AGCGGGCCCG
 AAGTATAACC TTTCTGGTG TTTACTCGAT TCGCCGGCGC